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SURVIVAL OF UNFED SQUID, *LOLIGO PEALEI*, IN AN AQUARIUM [1]

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Literature dealing with laboratory studies of live squid usually includes a statement that attempts to rear or maintain squid in aquaria are unsuccessful. When stated, the survival of squid in captivity is most often given for the longest lived individual and predictive survival rates are not specified. A considerable body of current research (principally that dealing with squid "giant axons") depends on a reliable supply and short term maintenance of squid. Several aspects of squid biology lag for want of effective means to carry out lengthy laboratory studies. Preparatory to possible future studies on the maintenance of the squid, *Loligo pealei* (Lesueur, 1821), we have observed squid survival when subjected to standardized conditions. Quantitative data were obtained in an attempt to specify survivorship for comparison with other reports and for evaluation of certain environmental factors.

The most dramatic results of attempts to rear cephalopods were reported by Choe (1966), who succeeded in fostering a few individuals of three species for more than 100 days and one loliginid squid for over 40 days. Edward T. LaRoe (University of Miami, unpublished) has repeatedly reared *Sepioteuthis sepioidea* to near maturity. Adults of this species have been maintained in floating pens for two months and possibly longer (Arnold, 1965). Immature squid, *Loligo vulgaris,* were reported to survive captivity for several weeks (Bidder, 1950) and for two months (Tardent, 1962). Tardent (1962) stated that sexually mature *L. vulgaris* do not normally live more than two weeks in the Naples Aquarium. Bidder (1950) reported that *L. vulgaris* is less sensitive to handling than *Loligo forbesi* and quoted a Dr. Sereni in the opinion that *L. pealei* at Woods Hole is far hardier and more resistant to handling than *L. vulgaris* at Naples. Roper (1965) reported a female *Doryteuthis plei* which survived 18 days in an aquarium, laying two egg masses in that time. Arnold (1962) gives the survival of sexually mature *L. pealei* as about five days and one instance of a male surviving for 17 days. These survival times refer to the longevity of the last remaining individual. In all of the references cited, squid were fed regularly and provided with running seawater. Only references dealing with loliginid squid have been cited because the survival of other families of cephalopods may not be comparable due to differences in life habits and ecological tolerances.

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Methods

Squid survival studies were conducted from mid-May through September, 1969, at the Marine Biological Laboratory, Woods Hole, Massachusetts. Seawater temperatures in the experimental aquarium were near ambient for the nearby squid habitats and ranged from 14.4 to 23.2° C; the maximum temperature occurred in late August. Live squid were captured by a floating fishtrap and by otter trawl nets The fishtrap was located off Quissett Harbor in Buzzards Bay and was operated intermittently during the summer months by Mr. Charles L. Wheeler of the Bureau of Commercial Fisheries, Biological Laboratory (Woods Hole). Trap hauls were made approximately every other day, usually in the morning. Otter trawl procedures were essentially the same as previously described (Summers, 1968). The #35 otter trawl cited was modified by replacement of the rubber rollers on the foot rope with a chain, exchange of the cod end liner for one with a stretched mesh dimension of 1.3 cm and use of trawl doors weighing 140 kg each. Most of the trawling was done with a 45–65 Long Island Sound balloon trawl made by George W. Wilcox Company, Mystic, Connecticut. The 45–65 trawl was narrower and taller than the #35 trawl, but utilized the same mesh dimensions, fittings and trawl doors. Trawling was restricted to midday tows in 10 to 30 m depths within a 20 km radius of Woods Hole (*i.e.*, Buzzards Bay, Vineyard Sound and western Nantucket Sound).

Trap caught squid were dip netted into tubs in a trap skiff and transferred to a cylindrical wooden tank of approximately 900 liter capacity aboard the R/V Blueback. Running seawater was pumped directly into this tank. The Blueback normally returned to the dock in 20 to 30 minutes where the remaining live squid were promptly dip netted into tubs and wheeled to the experimental tank on a cart. A final selection was made for apparently healthy squid before initiating the experimental procedure. Trawl caught squid were separated from the remainder of the catch in a periodically flushed, seawater filled tub and transferred to a conical fiberglass tank of approximately 400 liter capacity on the deck of the R/V A. E. Verrill. Running seawater was pumped directly into this tank. The conical shape effectively dampened water movements during vessel operations. Dead and moribund squid were periodically removed because the Verrill made from two to four trawl tows of 15 to 30 minutes each and sometimes took as long as 1½ hours to return to the dock. Handling at the dock was similar to the procedure for trap caught squid.

The experimental aquarium was kindly furnished by Mr. C. O. Schweidenback of the Marine Biological Laboratory. It was a rectangular, paraffin coated, concrete tank with inside dimensions of 98 by 138 cm and 65 cm deep. An overflow drain located near one corner kept the water depth at 45 cm giving a seawater volume of approximately 600 liters. A small piece of netting was anchored opposite the drain as a substratum for egg deposition. A copious supply of seawater was provided by an inflow hose located at the center of the tank, just below the water surface. A corrugated, green fiberglass cover with a single opening for the seawater inlet was fitted over the tank. During the summer months an airstone was placed in a vacant corner of the tank for additional aeration. Constant illumination was provided by a 75 watt spotlight set 25° off the vertical and shown directly on the center of the tank cover from a distance of

140 cm. This produced a uniform, diffuse light in the tank which was little affected by room lights and activities near the tank. The experimental tank was occasionally drained and hosed down.

Constant illumination was chosen for these runs because light was required for observations and because *L. pealei* is demersal in daylight hours (Summers, 1969 and unpublished results). The value of this choice was demonstrated in an aborted run when, by mistake, the tank was left uncovered with the light off for a few minutes. Several squid jumped out of the tank and those remaining were found swimming actively.

Experimental runs were made by placing a group of squid in the tank and observing survival for up to ten days at a time. Squid were checked at least once a day and usually three times daily; dead squid and the major portion of any fresh egg mass were removed and the water temperature was recorded. The number, mantle length, sex and maturity of dead squid were noted with pertinent observations at each inspection. No attempt was made to duplicate the initial numbers of squid in the tank from run to run because the supply of healthy animals varied and strict replicate experiments were impossible with one tank.

Squid characteristics varied with both season and method of capture. Sex ratios were close to one-to-one in all cases. Trawl caught squid ranged in size from 9 to 28 cm dorsal mantle length with median sizes of approximately 15 cm throughout the study period. Sexually immature individuals were not common among trawl caught squid except for one group in mid-August. Trap caught squid ranged in size from 5 to 28 cm mantle length with an overall median size of 8 cm. The median size of trap caught squid decreased through the study period and, with the exception of the first trap haul in mid-June, these squid were predominantly sexually immature. Thus, the two methods of capture appear to have sampled different portions of the squid population except early in the summer when the mature squid come close inshore to breed (see Verrill, 1882; Summers, 1968 and 1969).

Squid in these experimental runs were purposely not fed for a number of reasons. We felt that feeding might stimulate competition and promote tank contamination. Attempts to feed the squid would produce additional disturbances and could not readily be regulated so that every squid received a share of the food. The logistics of catching and holding live food organisms were other considerations in our decision. Finally, the value of feeding was a significant question in itself. Bidder's reports on the rapid digestion of squid (1950 and 1966) led us to believe that they might quickly starve and we were prepared to change our experimental procedure if necessary. On the other hand, we were interested in the survival of the squid over the first few days in captivity and chose not to feed them for simplicity in experimental design.

Results

Twelve experimental runs were continued until the last squid died or for a period of at least one week. Eight runs used trawl caught squid and four, trap caught squid; these were interspersed and randomly ordered according to availability. Experimental runs with trawl caught squid were generally begun with larger numbers of animals because trawl catches were larger than trap catches.

Survival of squid was averaged for the last observation in every 24 hour period for all runs resulting from a single method of capture. Figure 1 shows how the mean survival decreased with time and that the relative variation (standard deviation divided by the mean) increased with time for both trawl and trap caught squid. The mean survival of squid is shown on an absolute scale because the numbers of squid per run were kept within practical laboratory levels and there were insufficient runs, in our opinion, to justify the use of a relative scale. The observed mean survival of squid appears to be proportional to the number of squid in the tank and similar for both methods of capture. The survival rate does not vary appreciably with time in the tank within one week. Least square fit proportional survival curves are shown as lighter lines in Figure 1, these correspond closely to a survival rate of 71% per day (*i.e.,* 50% every two days). More exactly, it was 70.2% per day for trawl caught squid and 71.5% per day for trap caught squid.

Breeding activity and subsequent egg deposition is thought to cause rapid mortality in several species of cephalopods and is especially well documented for *Loligo opalescens* (McGowen, 1954; Limbaugh and Shepard, 1957; Hobson, 1965; Fields, 1965). Thus, survival of immature squid versus breeding, sexually mature squid as well as survival of males versus females in the latter group is of interest. The similarity of relative survival rates for trawl caught squid (sexually mature and breeding) and trap caught squid (sexually immature) shown in Figure 1 indicates that no major differences exist between these groups over a period of one

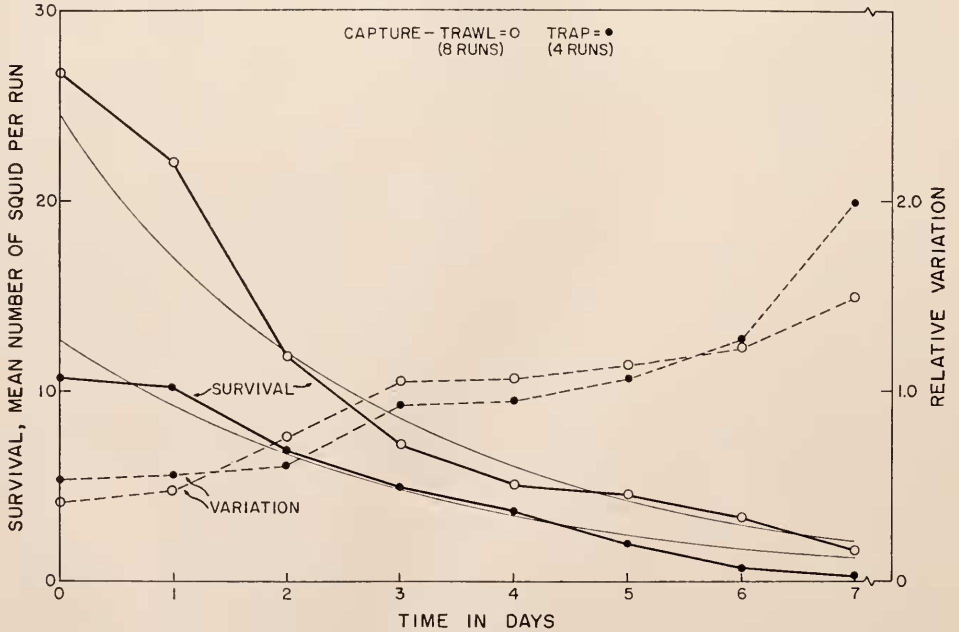

FIGURE 1. Mean survival of unfed squid, *L. pealei,* in an aquarium over a period of one week. Dashed lines represent the relative variation (standard deviation divided by the mean). Lighter lines are least square fit survival curves for the data and have proportional survival rates of 70.2% per day for trawl caught squid and 71.5% per day for trap caught squid.

week. Observations of dead or moribund squid associated with egg masses as reported for *L. opalescens* generally have no counterpart in *L. pealei* in either our field or laboratory experience.

Average mortalities illustrating sex and size factors are shown for selected runs employing trawl caught squid in Figure 2. The mortality of female squid shown in Figure 2A is based on four runs in which the numbers of sexually mature males and females were approximately equal. These data are contrasted with 49% of the total (unsexed) mortality (corresponding to the overall proportion of females in the runs). Use of a chi-square test failed to demonstrate significant differences between the observed and proportional mortalities of female squid over the period of one week.

Possible differential mortality of squid as a function of size was of concern in view of the literature on squid maintenance previously cited. The mortality of squid larger than the median size for each run (*i.e.*, larger than about 15 cm dorsal mantle length) shown in Figure 2B is based on seven runs containing predominantly sexually mature squid. These data are contrasted with 42% of the total mortality (corresponding to the overall proportion of larger squid in the runs). Use of a chi-square test failed to demonstrate significant differences between the observed and proportional mortalities of larger squid over the period of one week.

The results given above do not show effects related to water temperature; this varied almost 9° C during the experimental period, but never as much as 2° C

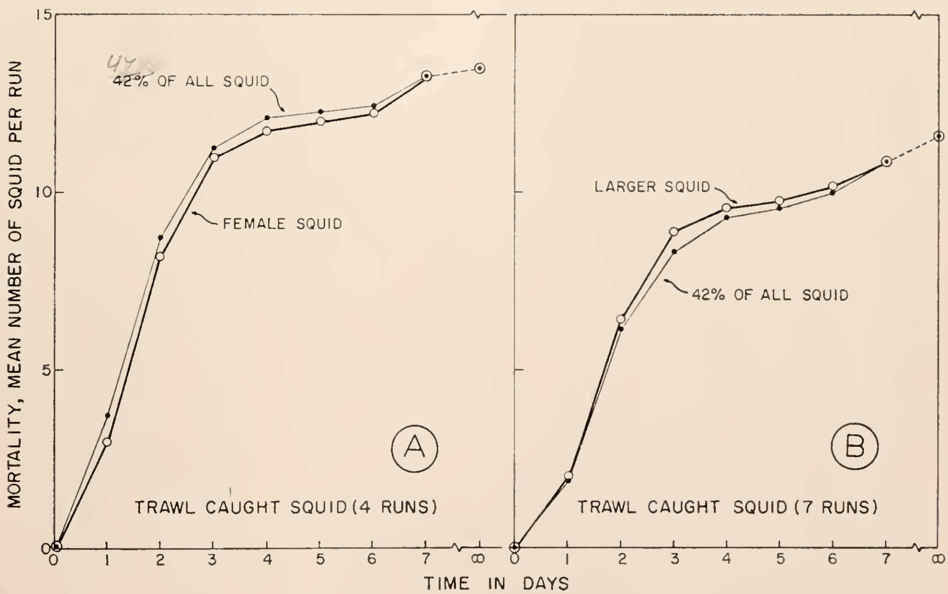

FIGURE 2. Mean mortalities of sexually mature, trawl caught squid, *L. pealei*, for selected runs over a period of one week. In Figure 2A, the mortality of female squid is contrasted with 49% of the total mortality (corresponding to the proportion of females) in four runs having approximately equal numbers of males and females. In Figure 2B, the mortality of larger squid (larger than the median mantle length) is contrasted with 42% of the total mortality (corresponding to the proportion of larger squid) in seven runs. Neither contrast is significant through the use of chi-square tests.

during any one run. In general, changes in water temperature were gradual and the animals were not subjected to water temperatures widely different from those occurring in nearby squid habitats. Although no optimum water temperature is implied, the three numerically most successful survivorships occurred at temperatures between 19.6 and 22.3° C. One run with an initial number of 40 squid and temperatures of 19.4 to 20.0° C, however, had no survivors on the fourth day.

Some notion of the probable course of any particular run was gained by an empirical evaluation of the initial survival rate. The observed number of survivors was compared with a calculated number based on the initial number of squid and the average survival rate of 71% per day. A survival trend was considered positive when the observed number was larger than the calculated value rounded to the nearest whole number. Five of the six runs with large initial numbers (21–40 squid) showed agreement in trends for both two and six day survivals. These six runs were equally divided positive and negative on the sixth day. Five of the six runs with small initial numbers (4–16 squid) had positive trends at two days, but agreement in trends for both two and six day survivals occurred in only one of the six runs. Eight of the twelve runs had negative survival trends on the sixth day. (In fact, five of the twelve runs had reached 100% mortality by the end of the sixth day.) Squid survived a full six days in runs with initial numbers of 39 (9 survivors), 30 (8 survivors), 30 (8 survivors), 16 (2 survivors), 31 (1 survivor), 21 (1 survivor) and 8 (1 survivor).

Discussion

The results presented here offer little that will optimize the maintenance of the squid, *L. pealei,* by way of selecting for a certain source, size or sex. Water temperatures between 14.4 and 23.2° C appear to be unimportant for laboratory survival. Survival for several days is numerically best with a large initial number of squid. Short term survival (*e.g.,* two days) is relatively better with small initial numbers of animals.

It was anticipated that survival would be influenced by several independent sources of mortality and that some of these would produce distinctive indentifiable trends. We anticipated that natural mortality from aging and related causes excluding predation would act at a low, constant rate which would be insignificant in our results. Our experimental method subjected squid to starvation and we expected a delayed, dramatic mortality during the run. In fact, no such mortality was observed even in runs which lasted ten days or longer. Although cannibalism of dead or dying squid was apparent in some cases, we cannot conclude that it was ever of sufficient quantity to sustain the surviving squid. We consider it unlikely that starvation was a major cause of mortality over a period of one week.

Persistent capture trauma cannot be eliminated as a cause of mortality in our experiments. Although the squid were carefully selected at the beginning of each experimental run, it is possible that some of them suffered an early death as a result of capture or handling injuries. We expected that this effect would result in a high initial mortality. We have noted that the proportion of squid surviving trawl capture appears to be inversely related to the quantity of the total catch and especially sensitive to the presence of rough surfaced invertebrates, sharks and fishes in the catch. We could not correlate laboratory survival of trawl caught squid with capture conditions and believe that the selection procedure effectively minimized the carryover of capture injuries. Furthermore, trap caught squid should have been free from capture damage, but not possible handling injuries. Figure 1 shows that the initial survival of trawl and trap caught squid followed the same relative pattern, therefore, we cannot credit capture trauma as a major cause of mortality.

Confinement of squid in the experimental tank promoted possible injury through unnatural and continual interaction with mechanical barriers. We anticipated that resulting mortalities would occur randomly with time and in proportion to the crowding of animals in the tank. We thought that crowding would increase the general level of activity and increase the probability that squid would injure themselves on the tank walls. Mortalities would follow injuries by some time which could not be specified, but which was considered variable and brief compared to the experimental run. Thus, we expected that confinement mortality would be roughly independent of confinement time and proportional to the number of squid. The observed results readily fit this model and we accept it as an important element among the causes of mortality in aquarium held squid.

The proportional survival model has several counterparts in familiar physical systems. Examples include radioactive decay and the kinetics of a first order chemical reaction. The response of an electronic lag network or mechanical spring dashpot system to step inputs are also analogous. Such transient phenomena are commonly described in control system theory with Laplace transformations. In examples where discrete units make up the particular assemblage under consideration, these are treated as statistically identical, each with an intrinsic probability for the subject event irrespective of all others. Although we recognize differences between the units in our study (individual squid), we have not succeeded in demonstrating that these are significant in laboratory survival. Hence, the proportional survival model is appropriate until separate causes of mortality can be isolated. The survival model can be expressed in the following formula: $n = n_0(R)^t$. Where n is the instantaneous number of surviving squid, n_0 is the initial number of squid, R is the average, daily survival rate (0.71 in our experiments) and t is the time in days. This formula is a special case of a more general class of equations having the form: $n = n_0(e^{-kt})$. Where k is a rate constant defined as follows: $k = \log_e(1/R)$.

Our results compare with those reported by Arnold (1962 and personal communication). Using four to ten, sexually mature *L. pealei* (mantle lengths of 15 to 25 cm) per run in a tank approximately 50% the capacity of ours, Arnold reported an average survival of about five days. Assuming his average run began with a middle number of squid (seven), that one squid (on an average) survived to the end of the fifth day and that survival rates in his runs were uniformly proportional, one can calculate a daily survival rate of 68% per day from his data. This is close to the 71% per day which we observed and may indicate that feeding (in Arnold's experiments) does not improve the short term survival rate.

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Summary

1. This paper reports the survival rate of unfed squid, *Loligo pealei*, in a 600 liter aquarium with running sea water and constant illumination.

2. Squid captured by a floating fishtrap and by otter trawl nets survived at the average rate of approximately 71% per day over a period of one week apparently independent of time in the tank, method of capture, water temperature, size or sex.

3. Initial numbers of squid between four and forty did not greatly alter the relative results. The early results, in conjunction with the survival rate given above and the initial number of squid, were useful in empirically evaluating the probable course of an experimental run.

4. It is possible that observed mortalities resulted from crowding and mechanical confinement. Other anticipated causes of mortality (*i.e.*, capture trauma and starvation) were not obvious in our results.

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